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Original Article

# SPECTROPHOTOMETRIC METHODS BASED ON CHARGE TRANSFER COMPLEXATION REACTIONS FOR THE DETERMINATION OF AMISULPRIDE IN PURE FORM AND PHARMACEUTICAL FORMULATIONS

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**ABSTRACT**

Objective*:* Two simple, accurate and precise spectrophotometric methods have been developed for the determination of amisulpride in pure form and pharmaceutical formulation (tablets).

Methods: The proposed methods were based on the charge transfer complexation reaction of amisulpride as 'n' electron donor with chloranilic acid (*p*-CLA) and 7,7,8,8-tetracyanoquinodimethane (TCNQ) as π acceptors to give highly coloured complex species. The coloured products were quantitated spectrophotometrically at 533 and 839 nm for *p*-CLA and TCNQ, respectively. Optimization of the different experimental conditions were studied.

Results: Beer's law was obeyed in the concentrations ranges of 10-130 and 2.0-32 µg $mL^{-1}$ for *p*-CLA and TCNQ, respectively with good correlation coefficient was ≥ 0.9998 with a relative standard deviation (R.S.D.) of ≤ 1.23%. The molar absorptivity, Sandell sensitivity, detection and quantification limits were also calculated. The developed methods were successfully applied for determination of amisulpride in tablets with good accuracy and precision and without interferences from common additives by applying the standard addition technique.

Conclusion*:* The developed methods have been validated statistically for their accuracy, precision, sensitivity, selectivity, robustness and ruggedness as per ICH guidelines and the results compared favourably with those obtained using the reported method.

**Keywords***:* Amisulpride; Charge transfer complexes; Spectrophotometry; Pharmaceutical formulation.

## INTRODUCTION

Amisulpride is a substituted benzamide chemically designated as 4-amino-N-[[(2RS)-1-ethylpyrolidin-2-yl]methyl]-5-(ethylsulphonyl)-2-methoxybenzamide [1-3] (Figure 1). It is classified as a second generation (atypical) antipsychotic. It is reported to have a high affinity for dopamine $D_2/D_3$ receptor antagonist, mainly used in the management of psychoses such as schizophrenia. Amisulpride is absorbed from GIT but the bioavailability is reported to be only about 43 to 48%. Plasma protein binding is reported to be low, metabolism is limited, with most of a dose appearing in the urine and faces as unchanged drug. The terminal half-life is about 12 hours [4].

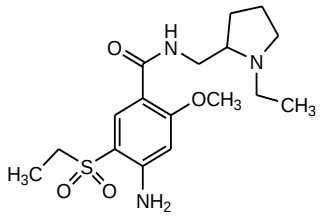



**Fig. 1: The chemical structure of amisulpride**

Amisulpride is official in British pharmacopoeia (BP) [3]. A literature survey revealed that there are various analytical methods have been reported for the determination of amisulpride in pharmaceutical dosage forms, which include non-aqueous titration [3], reversed phase high pressure liquid chromatography (RP-HPLC) [5-15], capillary electrophoresis (CE) [16] and electrochemical methods [17]. However, these methods are expensive and not available at most quality control laboratories. For routine analysis of the studied drugs, a simple, rapid and cost effective analytical method was required. The spectrophotometric technique continues to be the most preferred method for the assay of different classes of drugs in pure form, pharmaceutical formulations and biological samples, for its simplicity and reasonable sensitivity with significant economic advantages. There are few spectrophotometric methods have been developed for the estimation of amisulpride in dosage forms [18-23] (Table 1).

These methods were associated with some major drawbacks such as decreased selectivity due to measurement in ultraviolet region and/or decreased simplicity of the assay procedure. For these reasons, it was worthwhile to develop a new simple and selective spectrophotometric method for the determination of amisulpride in its pharmaceutical dosage forms.

The molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge transfer complexes, which absorb radiation in the visible region [24]. A variety of electron donating compounds have been reported to yield charge-transfer complexes with various π-acceptors [25-28].

The aim of the present study was directed to investigate simple, direct, sensitive, normal cost and precise spectrophotometric methods for simultaneous determination of amisulpride as a good n-electron donor via charge transfer complexation with π –acceptors; chloranilic acid (p-CLA) and 7,7,8,8-tetracyanoquinodimethane (TCNQ) as chromogenic reagents in pure form and its dosage forms (tablets). The reaction conditions of the methods have been established. In addition, the molar ratio of reactants was determined. No interference was observed in the assay of amisulpride from common excipients in levels found in pharmaceutical formulations. These methods are validated by the statistical data.

## MATERIALS AND METHODS

**Apparatus:** All absorption spectra were made using double beam Unikon 930 spectrophotometer (Kontron Instruments, Munchen, Germany) with a scanning speed of 200 nm/min and a band width of 2.0 nm, equipped with 10 mm matched quartz cells.

### Materials and Reagents

All chemicals and reagents used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily.

**Table 1**: **Comparison between the reported spectrophotometric methods for determination of amisulpride**

| | Method | λ max (nm) | Beer's law (µg mL$^{-1}$) | LOD (µg mL$^{-1}$) | molar absorptivity (L mol$^{-1}$ cm$^{-1}$) | References |
|---|---|---|---|---|---|---|
| 1. | Sodium nitrite and hydrochloric acid: a. Ethyl acetoacetate | 438 | 1.0-24 | 0.19 | 3.6918x10$^4$ | [18] |
| | b. 1-benzoyl acetone | 446 | 1.0-24 | 0.36 | 3.1557x10$^4$ | |
| | c. 8-hydroxyquinoline | 545 | 1.0-20 | 0.16 | 3.8146x10$^4$ | |
| 2. | sodium nitrite and hydrochloric acid: a. N-(1-naphthyl) ethylene-diamine dihydrochloride | 523 | 2.5 - 12.5 | | | [19] |
| | b. Diphenylamine | 382 and 490 | 5 - 25 and 10 - 50 | | | |
| | c. β-naphthol | 527 | 4 - 20 | | | |
| | d. Resorcinol | 521 | 2.5 - 12.5 | | | |
| | e. Chromotropic acid | 486 | 5 - 15 | | | |
| 3. | Sodium nitrite in acid medi: a. Phloroglucinol | 418.5 | 2.0-10 | 0.066 | 1.8 x 10$^4$ | [20] |
| | b. Resorcinol | 403.8 | 4.0-20 | 0.135 | 1.5 x 10$^4$ | |
| 4. | a. Bratton-Marshall | 525 | 1-5 | 0.028938 | 3.731×10$^5$ | [22] |
| | b. $FeCl_3$/ MBTH | 580 | 2-12 | 0.433523 | 2.725×10$^4$ | |
| | c. Folin-Ciocalteau phenol's | 625 | 10-50 | 0.17633 | 1.112×10$^4$ | |
| 5. | a. Methyl orange | 420 | 5.0-25 | 0.17633 | 1.112 x 10$^3$ | [23] |
| | b. Chloranilic acid | 545 | 50-250 | 2.5116 | 7.278 x 10$^3$ | |

**Materials**

Pharmaceutical grade amisulpride was kindly supplied by Al-Andalus Medical Company, Cairo-Egypt. Amipride tablets, labeled to contain 50 mg amisulpride per tablet (Al- Andalus) were purchashed from local commercial markets.

**Standard drug solution**

A stock standard solution of amisulpride (500 µg mL$^{-1}$) was prepared by dissolving an exact weight (50 mg) of pure drug in 5.0 mL methanol and the volume was diluted to the mark in a 100 mL calibrated flask with acetonitrile. A stock solutions of amisulpride (100 µg mL$^{-1}$) and (5.0 x 10$^{-3}$ M) were prepared from suitable dilution of the stock standard solution. The stock solutions of drug are stable for a period of 3.0 days when kept in the refrigerator.

**Reagents**

Chloranilic acid (*p*-CLA), (Fluka, Switzerland) and 7,7,8,8-tetracyanoquinodimethane (TCNQ), (Aldrich Chem. Co., Milwaukee, USA); (1.0 x 10$^{-3}$M) solutions were freshly prepared as (1.0 x 10$^{-3}$ M) in acetonitrile. The solutions were stable for at least one week at 4 ºC.

**General Procedures**

Into 10-mL calibrated flasks were placed (0.2-2.6 mL) and (0.1-1.6 mL) of (500 µg mL$^{-1}$) and (200 µg mL$^{-1}$) amisulpride solution using *p*-CLA and TCNQ methods, respectively in acetonitrile. Volumes 2.0 and 1.0 mL of (1.0 x 10$^{-3}$M) *p*-CLA and TCNQ, respectively were added. The reaction mixture was heat in a water-bath at 60±5 ºC for 10 min. Cool and then dilute to volume up to 10 mL with acetonitrile and the absorbance was measured at 533 and 839 nm for *p*-CLA and TCNQ, respectively against a reagent blanks prepared in the same manner.

**Procedure for pharmaceutical formulations**

The contents of twenty amipride tablets (50 mg amisulpride per tablet) were crushed, finely powdered, weight out and the average weight of one tablet was determined. An accurate weight equivalent to 10 mg amisulpride were transferred into a 100-mL calibrated flask, dissolved in least volume of methanol with shaking for 5.0 min and filtered through a sintered glass crucible ($G_4$) to remove excipient in the powdered tablets. The filtrate was diluted to 50 mL with acetonitrile in a 50 mL measuring flask to give 200 µg mL$^{-1}$ stock solution of amisulpride. Aliquot of the cited solutions was taken and analyzed as described under the above recommended procedures for construction of calibration curves. For the proposed methods, the content of tablets was calculated using the corresponding regression equation of the appropriate calibration graph. The method of standard addition was used for the accurate determination of amisulpride contents.

**Stoichiometric Relationship**

The Job's method of continuous variation [29] was employed to establish the stoichiometry of the coloured products. A 1.0 x10$^{-3}$ M standard solution of amisulpride and a 1.0 x10$^{-3}$ solution of *p*-CLA and TCNQ were used. A series of solutions was prepared in which the total volume of drug and reagent was constant (2.0 mL). The drugs and reagents were mixed in various proportions and diluted in a 10-mL calibrated flask with acetonitrile solvent. Measure the absorbance at optimum wavelengths after treating each reagent at best time and temperature against a reagent blank following the above mentioned procedure.

**RESULTS**

**Absorption spectra**

In the present investigation, we investigate the development of simple, rapid, accurate, reproducible and adequately sensitive spectrophotometric methods for determination amisulpride in bulk powder and pharmaceutical formulation (tablets) based on the formation of charge-transfer complex of amisulpride as electron-donor with selected π-acceptors (*p*-CLA and TCNQ) in acetonitrile. They produce a new band of absorption intensity at 533 and 839 nm using *p*-CLA and TCNQ, respectively (Figures 2 and 3). which was characteristic for each complex (Tables 2). These new bands were used for a quantitative determination of amisulpride.

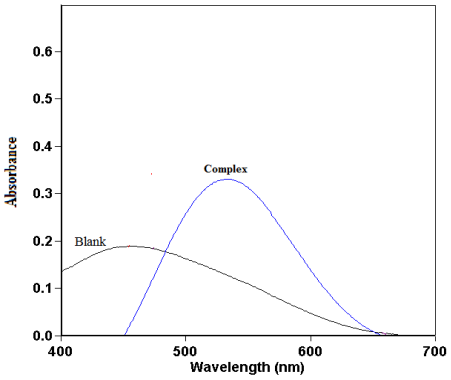



**Fig. 2: Absorption spectra of reaction products of 130 µg mL$^{-1}$ amisulpride solution with *p*-CLA (1.0 x 10$^{-3}$ M) in acetonitrile against blank solution.**

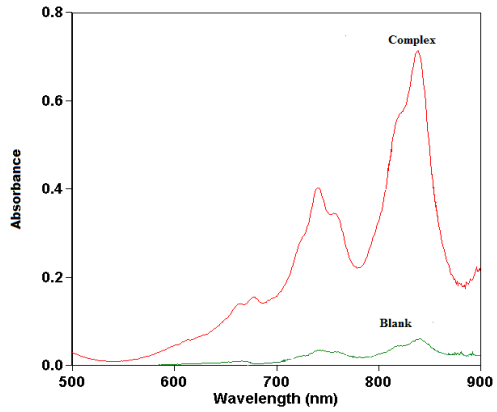



**Fig. 3: Absorption spectra of reaction products of 30 µg mL$^{-1}$ amisulpride solution with TCNQ (1.0 x 10$^{-3}$ M) in acetonitrile against blank solution.**

### Optimization of Reaction Conditions

The influence of different parameters on the colour development was studied to determine optimum conditions for the assay procedures.

### Effect of solvents

Different solvents such as acetone, methanol, ethanol, dichloromethane, 1,2-dichloroethane, acetonitrile and chloroform were examined. Acetonitrile was found to be the best solvent for all the reagents, because it has a high relative permittivity which ensures the maximum yield of *p*-CLA$^{\cdot-}$ and TCNQ$^{\cdot-}$ species. Of the other solvents examined, chloroform, acetone, dichloromethane and 1,2-dichloroethane are possible substitutes. The formation of *p*-CLA$^{\cdot-}$ and TCNQ$^{\cdot-}$ radicals was possible in methanol or ethanol, however, the colour intensity was lower than in acetonitrile.

### Effect of reagent concentration

The optimum concentrations that give maximum colour formation using 2.0 and 1.0 mL of (1.0 x 10$^{-3}$ M ) *p*-CLA and TCNQ solutions, respectively were found to be sufficient for the production of maximum and reproducible colour intensity in acetonitrile. Higher concentrations of the reagent did not affect the colour intensity (Figure 4).

### Effect of time and temperature

The optimum reaction time was determined by following the colour intensity at ambient temperature (25 ±2 °C). Complete colour development was attained after 10 min on raising the temperature on a water-bath to 60 ± 5 °C using both *p*-CLA and TCNQ methods. The colour remained stable for 4.0 h for *p*-CLA and TCNQ reagent complexes.

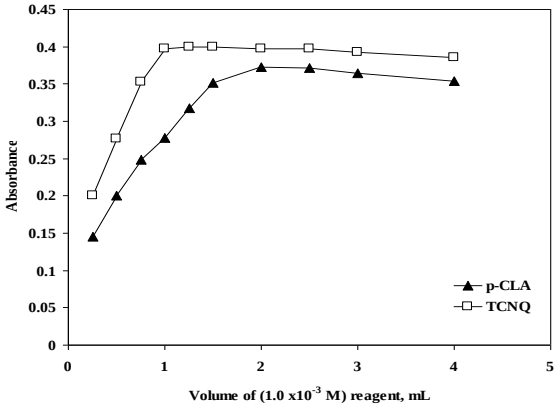



**Fig. 4: Effect of reagent concentration on the absorbance of charge transfer complexes formed between amisulpride and acceptors (1.0 x 10$^{-3}$ M)**

### Stoichiometry of the reaction

The stoichiometric ratio of the reactants (drug : reagent) was determined by Job's method [29] of continuous variation for the reaction between amisulpride and *p*-CLA and TCNQ reagents, which shows that the interaction occurs between an equimolar solution of the drug and the reagents. The result indicated that the charge transfer complex was formed in the ratio of 1:1 (Figure 5). On the basis of the literature data and our experimental results, tentative reaction mechanisms for amisulpride-TCNQ complex is proposed and given in Scheme 1, respectively.

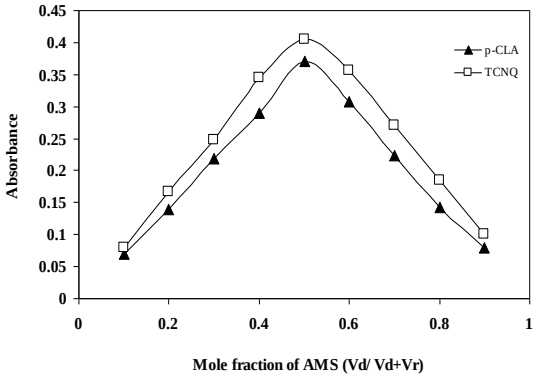


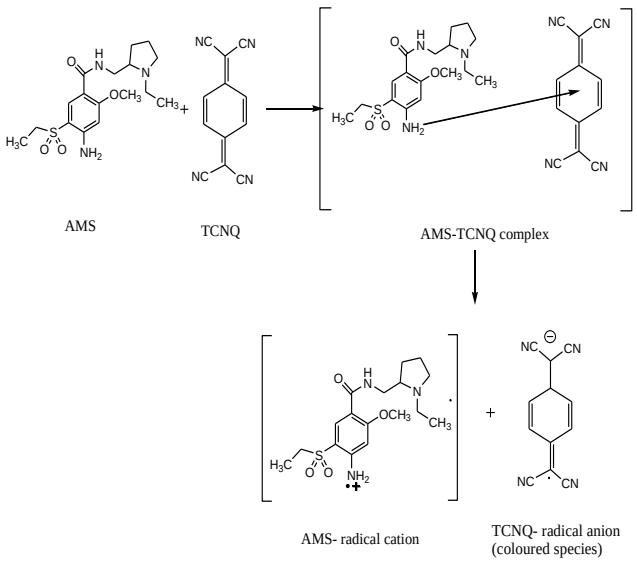

**Fig. 5: Continuous variation plots for the reaction of amisulpride with *p*-CLA and TCNQ. λ= 533 and 839 nm, respectively. Total molar concentration = 1.0 x 10$^{-4}$ mol L$^{-1}$.**

### Method validation

Validation of the described methods for assay of bulk amisulpride was examined via linearity, sensitivity, precision, accuracy, repeatability, reproducibility, selectivity, and robustness according to ICH guidelines [30].

### Linearity and sensitivity

Under the optimum conditions a linear correlation was found between absorbance at $\lambda_{max}$ and concentration of amisulpride in the ranges of 10-130 µg mL$^{-1}$ and 2.0-32 µg mL$^{-1}$ using *p*-CLA and TCNQ methods, respectively. The calibration graph is described by the regression equation:




**Scheme 1: Proposed reaction pathway for the formation of charge transfer complex between amisulpride and TCNQ.**

A = a + b C (1)

(where A = absorbance, a = intercept, b = slope and C = concentration in µg $mL^{-1}$) obtained by the method of least squares [31]. Correlation coefficient, intercept and slope for the calibration data are summarized in Table 2. For accurate determination, Ringbom concentration range [32] was calculated by plotting log concentration of drug in µg $mL^{-1}$ against transimittance % from which the linear portion of the curve gives accurate range of microdetermination of amisulpride and represented in Table 2. Sensitivity parameters such as apparent molar absorptivity (ε) and Sandell's sensitivity (SS) values, the limits of detection and quantification were calculated as per the current ICH guidelines (ICH) [30], are illustrated in Table 2. The high molar absorptivity and lower Sandell sensitivity values reflect the good and high sensitivity of the proposed methods. The validity of the proposed methods was evaluated by statistical analysis [33] between the results achieved from the proposed methods and that of the reported methods. Regarding the calculated Student's *t*-test and variance ratio *F*-test (Table 2), there is no significant difference between the proposed and reported method [8] regarding accuracy and precision. The limit of detection (LOD) is defined as the minimum level at which the analyte can be reliably detected for the drug was calculated using the following equation [30, 33] and listed in Table 2:

LOD = $3s / k$

where $s$ is the standard deviation of replicate determination values under the same conditions as for the sample analysis in the absence of the analyte and $k$ is the slope of the calibration graph. In accordance with the formula, the limits of detection were found to be 2.92 and 0.46 µg $mL^{-1}$ using *p*-CLA and TCNQ, respectively. The limits of quantification, LOQ, is defined as the lowest concentration that can be measured with acceptable accuracy and precision[30, 33],

LOQ = $10 s / k$

According to this equation, the limit of quantification was found to be 9.73 and 1.53 µg $mL^{-1}$ using *p*-CLA and TCNQ, respectively.

**Table 2: Analytical and regression parameters of proposed oxidation spectrophotometric methods for determination of amisulpride**.

| Parameters | *p*-CLA | TCNQ |
|---|---|---|
| Wavelengths $\lambda_{max}$ (nm) | 533 | 839 |
| Solvent | Acetonitrile | Acetonitrile |
| Color stability | 4.0 | 3.0 |
| Beer's law limits (µg $mL^{-1}$) | 10-130 | 2.0-32 |
| Ringbom optimum concentration range (µg $mL^{-1}$) | 20-115 | 4.0-30 |
| Molar absorptivity ε, (L/$mol^{-1}$ $cm^{-1}$) x $10^4$ | 0.1143 | 0.440 |
| Sandell·s sensitivity (ng $cm^{-2}$) | 323.27 | 83.98 |
| Regression equation [a] | | |
| Slope (b) | 0.0029 | 0.0117 |
| Intercept (a) | 0.0036 | 0.0006 |
| Correlation coefficient (*r*) | 0.9998 | 0.9998 |
| Mean ± SD | 99.73 ± 0.98 | 99.52 ± 1.17 |
| Relative standard deviation, RSD% | 0.98 | 1.18 |
| Relative error, RE% | 1.03 | 1.23 |
| LOD, (µg $mL^{-1}$) [b] | 2.92 | 0.46 |
| LOQ, (µg $mL^{-1}$) [b] | 9.73 | 1.53 |
| Calculated *t*-value [c] | 0.376 | 0.06 |
| Calculated *F*-value [c] | 1.31 | 1.09 |

[a] $A = a + bC$, where $C$ is the concentration in µg $mL^{-1}$, $A$ is the absorbance units, $a$ is the intercept, $b$ is the slope.[b] LOD, limit of detection; LOQ, limit of quantification; ε, molar absorptivity. [c] The theoretical values of $t$ and $F$ are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom ($p$= 0.05).

**Accuracy and precision**

The accuracy and precision of the methods (within-day and between-days) were evaluated by performing six replicate analyses on pure drug solution at three different concentration levels (within the working range). Percentage relative standard deviation (RSD%) as precision and percentage relative error (RE%) as accuracy of the proposed spectrophotometric methods were calculated. The relative standard deviation (RSD) values were ≤ 1.40% in all cases, indicating good repeatability of the suggested methods. This level of precision of the proposed methods was adequate for the quality control analysis of the studied drugs. The percentage relative error (RE %) calculated using the following equation:

$$\% \, R.E. = \left[ \frac{found - taken}{taken} \right] \times 100$$

RE % is an indicator of accuracy in the range (-0.6-0.2) also indicating high accuracy and repeatability of the methods. The intra-day and inter-day precision and accuracy results show that the proposed methods have good repeatability and reproducibility reflecting the usefulness of the methods in routine analysis (Table 3).

**Table 3: Evaluation of intra-day and inter-day accuracy and precision for amisulpride obtained by the proposed methods**

| Methods | Added (µg $mL^{-1}$) | Recovery % | Precision RSD % [a] | Accuracy RE % | Confidence Limit [b] |
|---|---|---|---|---|---|
| | ***Intra-day*** | | | | |
| *p*-CLA | 25 | 99.80 | 0.75 | -0.20 | 24.95 ± 0.196 |
| | 50 | 100.20 | 1.08 | 0.20 | 50.1 ± 0.568 |
| | 100 | 99.40 | 1.34 | -0.60 | 99.4 ± 1.4 |
| TCNQ | 5.0 | 99.90 | 0.50 | -0.10 | 4.99 ± 0.03 |
| | 15 | 100.20 | 0.94 | 0.20 | 15.03 ± 0.148 |
| | 25 | 99.40 | 1.36 | -0.60 | 24.85 ± 0.355 |
| | ***Inter-day*** | | | | |

| | | | | | |
|---|---|---|---|---|---|
| *p*-CLA | 25 | 99.30 | 0.53 | -0.70 | 24.83 ± 0.138 |
| | 50 | 99.90 | 0.76 | -0.10 | 49.95 ± 0.398 |
| | 100 | 99.10 | 1.28 | -0.90 | 99.10 ± 1.331 |
| TCNQ | 5.0 | 99.70 | 0.62 | -0.30 | 4.99 ± 0.032 |
| | 15 | 99.50 | 0.81 | -0.50 | 14.93 ± 0.127 |
| | 25 | 100.10 | 1.40 | 0.10 | 25.03 ± 0.368 |

[a] Mean of six determination, RSD%, percentage relative standard deviation; ***RE***%, percentage relative error.[b] Mean ± standard error.

**Robustness and ruggedness**

The robustness of the methods was evaluated by making small incremental changes in the volume of reagent (± 0.2 mL) and time (±1.0 min), and the effect of the changes were studied on the absorbance of the charge transfer complex. The changes had negligible influence on the results as revealed by small intermediate precision values expressed as %RSD (≤ 3.0%). Method ruggedness was demonstrated having the analysis done by three analysts, and also by a single analyst performing analysis using three different spectrophotometer instruments in the same laboratory. Intermediate precision values (%RSD) in both instances were (≤ 2.0%) indicating acceptable ruggedness.

**Recovery studies by standard addition technique**

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. In this study, pre-analyzed tablet powder was spiked with pure drug at different concentration levels and the total was determined by the proposed methods using standard addition technique.

The percent recovery of pure amisulpride added was in the range 98.40–100.60% with relative standard deviation of 0.68–0.75 (Table 4) indicating that the recovery was good, and revealed that the co-formulated substances did not interfere in the determination. The results of recovery study are compiled in Table 4.

**Table 4: Application of the standard addition technique for the determination of amisulpride in amipride tablets using the proposed methods**

| Sample | Taken (µg mL$^{-1}$) | *p*-CLA | | Taken (µg mL$^{-1}$) | TCNQ | |
|---|---|---|---|---|---|---|
| | | Added (µg mL$^{-1}$) | Recovery [a] (%) | | Added (µg mL$^{-1}$) | Recovery [a] (%) |
| Amipride tablets | 10 | - | 99.20 | 2.0 | - | 99.10 |
| | | 20 | 99.00 | | 6.0 | 100.30 |
| | | 40 | 98.70 | | 12 | 99.70 |
| | | 60 | 98.80 | | 18 | 99.90 |
| | | 80 | 99.70 | | 24 | 100.60 |
| | | 100 | 100.50 | | 30 | 98.40 |
| Mean ± SD | | | 99.32±0.68 | | | 99.70±0.75 |
| R.S.D% | | | 0.68 | | | 0.75 |
| V | | | 0.47 | | | 0.57 |
| S.E | | | 0.278 | | | 0.306 |

[a] Average of six determinations.

**Interference studies**

The studied drug was determined in the presence of possible excipients and additives such as lactose, microcrystalline cellulose, sodium starch glycolate and magnesium stearate. Under the experimental conditions employed, to a known amount of drug, excipients in different concentrations were added and studied. Excipients do not interfere with the assay. In addition, recoveries in most cases were around 100% and the lower values of the RSD (≤ 2.0%) indicate the good precision of the methods. Regarding the interference of the excipients and additives usually presented in pharmaceutical formulation and interference due to the degradation products of amisulpride, the energy of the charge transfer ($E_{CT}$) depends on the ionization potential ($I_P$) of the donor and the electron affinity of the acceptor ($E_A$), hence the $\lambda_{max}$ values of the other π-donors mostly differ from that of the investigated compounds if they are able to form charge transfer complexes. Preliminary experiments showed that all additives, excipients and degradate products did not form charge transfer complexes with the studied acceptors indicating the high selectivity of the proposed methods and applicability to use for routine determination in pure and in dosage forms.

**Application of the proposed methods to the analysis of tablets**

In order to evaluate the analytical applicability of the proposed methods to the quantification of amisulpride in commercial tablets, the results obtained by the proposed methods were compared with those of the reference method [23] by applying Student's t-test for accuracy and F-test for precision. The results (Table 5) show that the Student's t- and F-values at 95% confidence level are less than the theoretical values, indicating that there is a good agreement between the results obtained by the proposed methods and the reference method with respect to accuracy and precision.

**Table 5: Application of the proposed methods for the determination of amisulpride in Amipride tablets using the proposed methods**

| Samples | Proposed methods | | Reported method [23] |
|---|---|---|---|
| | *p*-CLA | TCNQ | |
| Amipride tablets | | | |
| Recovery ± RSD [a] | 99.32 ± 0.68 | 99.70±0.75 | 100.12 ± 0.81 |
| *t-value* [b] | 1.69 | 0.85 | |
| *F*-value [b] | 1.42 | 1.17 | |

[a] Average of six determinations. [b] The theoretical values of *t* and *F* are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (*p*= 0.05).

**DISCUSSION**

Chloranilic acid (*p*-CLA) exists in three ionic forms, the neutral yellow- orange $H_2A$ at very low pH, the dark purple $HA^-$ which is stable at pH 3.0 and a colorless $A^{2-}$, which is stable at high pH; these transformations are illustrated in the following scheme:

$H_2A \rightleftharpoons H^+ + HA^-$(violet),

$HA^- \rightleftharpoons H^+ + A^{2-}$(colorless).

Since the interaction of amisulpride with *p*-CLA in acetonitrile forms charge transfer complex gave a violet product *(p*-CLA radical anion) which absorbing maximally at wavelength 533 nm, it might be concluded that $HA^-$ was the form of *p*-CLA involved in the reaction described herein. This compound is considered to be an intermediate molecular association complex which dissociates in the corresponding radical anions in acetonitrile solvent. Amisulpride reacts with TCNQ yields intense bluish-green colored radical anion ($TCNQ^{-}$) in acetonitrile, which exhibits strong absorption at 839 nm most probably due to the formation of charge-transfer complex between the drug acting as n-donor (D) or Lewis base, and TCNQ, as π-acceptors(A) or Lewis acids [24]:

$$D^{\bullet\bullet} + A \rightleftharpoons \underset{\text{DA complex}}{\left[D^{\bullet\bullet} \rightarrow A\right]} \xrightleftharpoons{\text{Polar solvent}} D^{\bullet+} + \underset{\text{radical anion}}{A^{\bullet-}}$$

The dissociation of DA complex is promoted by the high dielectric constant of acetonitrile. Further support for the assignment was provided by the comparison of the absorption bands with those of the *p*-$CLA^{-}$ and $TCNQ^{-}$ radical anions produced by the iodide reduction method.

**CONCLUSIONS**

The present study described the successful evaluation of two π acceptors (*p*-CLA and TCNQ) as analytical reagents in the development of simple and rapid charge transfer complexation spectrophotometric methods for the accurate determination of amisulpride in bulk drug and in its tablets and validated as per the current ICH guidelines. The present spectrophotometric methods are characterized by simplicity since they do not involve any critical experimental variable and are free from tedious, time-consuming, extraction steps and do not need expensive sophisticated apparatus unlike many previous methods. The proposed methods have additional advantages of ease of operation and possibility of carrying them out with a common laboratory instrument unlike many other instrumental methods reported for amisulpride. They are characterized by high selectivity and comparable sensitivity with respect to the existing methods. The accuracy, reproducibility, simplicity, and cost-effectiveness of the methods suggest their application in the quality control laboratories where the modern and expensive instruments are not available.

**Conflict of Interests**

The authors declare that they have no conflict of interests with the company name used in the paper.

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